
Homology-driven genome editing in hematopoietic stem and progenitor cells using ZFN mRNA and AAV6 donors.

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Public Summary:

Genome editing with targeted nucleases and DNA donor templates homologous to the break site has proven challenging in human hematopoietic stem and progenitor cells (HSPCs), and particularly in the most primitive, long-term repopulating cell population. Here we report that combining electroporation of zinc finger nuclease (ZFN) mRNA with donor template delivery by adeno-associated virus (AAV) serotype 6 vectors directs efficient genome editing in HSPCs, achieving site-specific insertion of a GFP cassette at the CCR5 and AAVS1 loci in mobilized peripheral blood CD34⁺ HSPCs at mean frequencies of 17% and 26%, respectively, and in fetal liver HSPCs at 19% and 43%, respectively. Notably, this approach modified the CD34⁺CD133⁺CD90⁺ cell population, a minor component of CD34⁺ cells that contains long-term repopulating hematopoietic stem cells (HSCs). Genome-edited HSPCs also engrafted in immune-deficient mice long-term, confirming that HSCs are targeted by this approach. Our results provide a strategy for more robust application of genome-editing technologies in HSPCs.

Scientific Abstract:

Genome editing with targeted nucleases and DNA donor templates homologous to the break site has proven challenging in human hematopoietic stem and progenitor cells (HSPCs), and particularly in the most primitive, long-term repopulating cell population. Here we report that combining electroporation of zinc finger nuclease (ZFN) mRNA with donor template delivery by adeno-associated virus (AAV) serotype 6 vectors directs efficient genome editing in HSPCs, achieving site-specific insertion of a GFP cassette at the CCR5 and AAVS1 loci in mobilized peripheral blood CD34⁺ HSPCs at mean frequencies of 17% and 26%, respectively, and in fetal liver HSPCs at 19% and 43%, respectively. Notably, this approach modified the CD34⁺CD133⁺CD90⁺ cell population, a minor component of CD34⁺ cells that contains long-term repopulating hematopoietic stem cells (HSCs). Genome-edited HSPCs also engrafted in immune-deficient mice long-term, confirming that HSCs are targeted by this approach. Our results provide a strategy for more robust application of genome-editing technologies in HSPCs.

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